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Research Article

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Simultaneous determination of four active components in Shengmai injection and its application to the quality control in productive process

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ABSTRACT

The purpose of the present work was to develop and validate a reliable and effective method for the analysis of Shengmai injection and its raw materials by reverse phase high pressure liquid chromatography. The Kromasil C18 column (5 μm, 4.6 mm × 250 mm)was used. Water containing 0.01% phosphoric acid and acetonitrile were used as mobile phases with gradient elution. The detection wavelengths was monitored at 203nm. The flow rate was set at 1.0 mL/min and column temperature was 35°C. ginsenosides Rg_1, Re, Rb_1 and schizandrin were used as marker compounds and determined for the quality control in productive process. On the basement of method validation, 5 samples of Shengmai injection and 3 batchs of raw materials were monitored for the quality control. The analytical method has suitable levels of precision, reproducibility, stability and linearity , which could be used as a new method for the quality control of Shengmai.

Keywords: Shengmai injection, RP-HPLC, ginsenosides, schizandrin

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INTRODUCTION

Shengmai Yin (Shengmai San) is a typical TCM prescription consisting of three different herbal components: Panax ginseng, Ohiopogon japonicus and Schisandra chinensis. Shengmai Yin has exact clinical efficacy in the treatment of various cardiovascular diseases, meanwhile it has been shown to have anti-inflammatory[1], anti-oxidative properties[2-4],neuroprotective effects[5]. Shengmai injection were developed based on the this ancient prescription "Shengmaiyin". It was prepared using modern technology to increase stability and shelf-life, which is a sterile aqueous solution that is obtained by these three fresh herbs.

Ginsenosides are considered as primary active ingredients of *Panax ginseng*, which exert direct depressant action upon cardiomyocytes contraction[6-7]. As the representative active lignans in *Schisandra chinensis*, schizandrin might be useful in treating memory deficits[8] and also have anti-oxidative activity[9].The concentration of ginsenosides and lignans in raw material herbs are variable, which is associated with different growing conditions, harvest season, age of the plant,and methods of extracting and processing. Therefore, ginsenoside Rg_1, Re, Rb_1 and schizandrin were choesn as representative quantity-control indicators of Shengmai injection. Chromatographic methods can be applied for the quality control of pharmaceutical preparations due to their many advantages such as high efficiency, high-speed and the possibility of their utilization in automatized systems[10-13]. A simple RP-HPLC method has been developed for routine analysis and quality control in productive process of the injection.

EXPERIMENTAL SECTION

Instrumentation

HPLC-DAD analysis was carried out using Agilent 1100 HPLC system equipped with quaternary gradient pump, a photodiode array detector ,vacuum degasser, an auto-sampler, column thermostat and Agilent ChemStation software.

KQ-100 ultrasonic cleaner was from Kunshan Ultrasonic Instrument Co., company; Milli-Q ultrapure water for preparation. electronic balance was from Sartorius Scientific Instruments (Beijing) Co., Ltd.

Chemical reagents
HPLC Grade Acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA), ultra pure water, Phosphoric acid (analytical grade) were obtained from Beijing Chemical Reagent Company.

Ginsenoside Rg_1 (lot number: 110703-200424), ginsenoside Re (lot number: 110754-200319), ginsenoside Rb_1 (lot number: 110704-200318), schizandrin (lot number: 110857-200304). All reference substance were obtained from National Institute for the Control of Pharmaceutical and Biological Products, China.

Shengmai injection preparations were commercial products (batch number: 120503，120504，120505，120506，120507).

Panax ginseng was collected from Jilin-Changbai Mountain, and *Schisandra chinensis* was from Heilongjiang

Chromatographic Conditions
Column was Kromasil C_{18} (5 μm, 4.6 mm × 250 mm); The mobile phase consisted of acetonitrile (A) and 0.01% aqueous phosphoric acid (B) in a binary gradient elution program follows: 0—20 min, 80% B—80% B; 20—40min, 64% B; 40—60 min, 35% B; 60—65 min, 80% B; The column temperature was maintained at 35ºC and the flow rate was 1.0 mL/min; detection wavelength was set at 203 nm.

Preparation of mixed standard solution
Reference substance ginsenoside Rg_1,Re,Rb_1 and schizandrin were accurately weighed and dissolved in 2 ml of methanol as a stock solution. The final concentration of the standard solution were 644.6, 482.8, 912.6 and 81.8μg/ml, respectively

preparation of Sample solution of injection
Shengmai injection were filtered through a. 0.22μm filter and 20μl were injected directly into the HPLC system.

preparation of Sample solution of herb
75% ethanol extracts of *Panax ginseng* and ethanol precipitation of total water extracts of *Schisandra chinensis* were extracted (0.1046 and 0.0109 g/ml, final concentration)in accordance with the production process of the injection. All extracts of raw material herbs was filtered before injection.

Precision
The stock solutions of reference substances were measured for five times under the same analytical conditions in a day. the relative standard deviation (RSD) values of retention time were all lower than 0.3%, while the RSD values of peak area were 0.48%, 0.91%, 0.73% and 0.59%, respectively.The results showed that the assay had a good intra-day precision.

Linearity
The calibration curve were obtained by plotting the concentration(μg/ml) as the x-axis versus peak areas on Y-axis and regression equations were computed. Six concentrations of the four Standards were analyzed. Calibration curves of all analytes showed good linearity over a wide concentration range. The test results were given in Table-1.

Table-1: Result of regression analysis on calibration curves

Compd	Regression equation	r^2	Linear range (μg/mL)
Ginsenoside Rg_1 Ginsenoside Re Ginsenoside Rb_1 Schizandrin	*Y*=6.4247 *X*-24.7737	0.9994	32.23-644.6
	Y=4.6796*X*-17.5092	0.9996	24.14-482.8
	Y=5.3502 *X*-27.6388	0.9997	45.633-912.6
	Y=97.5086 *X*-9.1615	0.9998	4.09-81.8

Accuracy
The accuracy of the method was determined by means of recovery experiments. Particular amount of the authentic standards were added into the pre-analyzed sample solution at three levels (80%, 100% and 120%, three replicates of each concentration).The mixed samples were analyzed using the developed HPLC method mentioned above.It was confirmed from recovery results that this developed method is highly accurate for the determination of these components. The data of accuracy study was given in Table-2.

Table-2: Recovery of four ingredients for quantitative analysis (*n* = 3)

Compd	Level of Addition%	Recovery(%)	RSD (%)
Ginsenoside Rg_1	80	99.13	1.95
	100	99.61	1.76
	120	100.82	1.93
Ginsenosides Re	80	100.29	2.13
	100	101.04	2.06
	120	100.65	2.24
Ginsenoside Rb_1	80	99.58	1.43
	100	99.23	1.97
	120	100.46	1.81
Schizandrin	80	99.54	1.72
	100	99.08	1.88
	120	100.27	2.01

Stability

For the stability testing, sample solution was stored at ambient temperature (25°C) for 24h. The same real sample was re-analyzed after 12 and 24h time intervals and compared against fresh sample. As shown in Table-3,the RSD values of the stability was less than 2.0%. It is illustrated that the solution was found to be stable within 24h during assay determination.

Table-3: Stability of four ingredients for quantitative analysis (*n* = 3)

Time intervals	Assay after 12h (RSD, %)	Assay after 24h (RSD, %)
	1.32	1.54
Ginsenoside Rg_1 Ginsenoside Re	1.87	1.96
Ginsenoside Rb_1 Schizandrin	0.89	0.93
	0.95	0.97

Sample analysis

The validated HPLC method was applied to the simultaneous determination of four active components in herbs and injection. The contents of Ginsenoside Rg_1,Re,Rb_1 and schizandrin were calculated by external standard method (Fig-1 and Table-4).

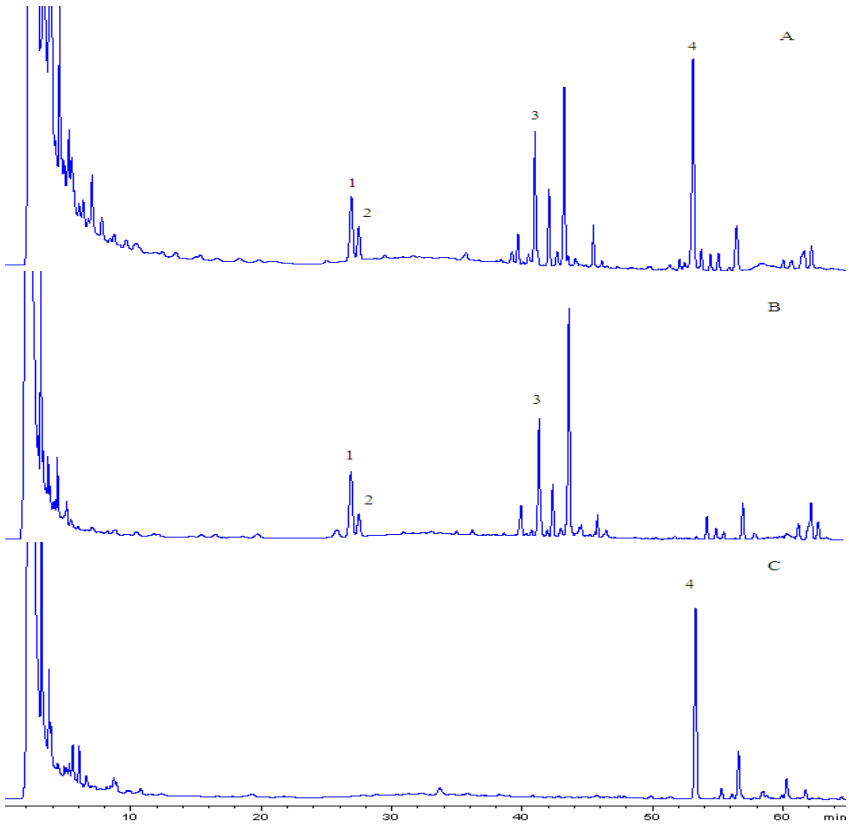

Fig 1: A – sample solution of injection; B - sample solution of Panax ginseng and C - sample solution of Schisandra chinensis. The peaks marked with 1–4 are: 1–Ginsenoside Rg_1; 2– Ginsenoside Re; 3– Ginsenoside Rb_1 and 4–schizandrin

Table-4: Determination of four constituents of samples

Sample	Content (μg/mL)			
	Ginsenoside Rg_1	Ginsenosides Re	Ginsenoside Rb_1	Schizandrin
Injection (120503)	120.96	75.18	188.57	17.83
Injection (120504)	123.18	76.72	190.41	19.05
Injection (120505)	119.51	74.91	187.69	17.02
Injection (120506)	122.35	76.03	189.26	18.68
Injection (120507)	117.84	73.96	186.53	16.23
***Panax ginseng* 1**	219.26	102.97	297.35	—
***Panax ginseng* 2**	214.38	100.54	295.12	—
***Panax ginseng* 3**	215.79	101.18	295.86	—
***Schisandra chinensis* 1**	—	—	—	40.19
***Schisandra chinensis* 2**	—	—	—	39.81
***Schisandra chinensis* 3**	—	—	—	38.95

CONCLUSION

The results demonstrated that the proposed HPLC method is simple, sensitive, precise and reproducible. It can be successfully applied for quantification of ginsenosides Rg_1, Re, Rb_1 and schizandrin in Shengmai injection.The method was also used for the quality control in the productive process from herbs and intermediate products to herbal preparation. The determination of raw plant material can be used to confirm the stability of the production technology and further ensure the quality of the finished product. In addition, the chemical fingerprints of multi-components were obtained in one run, and therefore can be readily utilized as a comprehensive quality control of various Shengmai products.

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